ENGINEERING OF ANTIMICROBIAL BIOPLASTICS FROM INVASIVE ALGAE CAULERPA PROLIFERA, UNDARIA PINNATIFIDA, AND WASTE CORN COBS

A Science Research Project

Presented to

SARSEF Science and Engineering Fair

by

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ABSTRACT

Are biodegradable plastics in the market truly biodegradable? Current biodegradable plastics such as PLA and oxo-biodegradables are found to be inefficient for they require a special composting facility to biodegrade in which most individuals do not have access to. To solve this problem, this project tried to find ways to produce a bioplastic using invasive algae and waste corn cobs.

This project was conducted using the following methods. First, extraction of starch from waste corn cobs. Second, creating Sodium alginate from Undaria pinnatifida. Next, to obtain the extracts from Caulerpa prolifera and common herbs, ethanol extraction and rotary evaporation were performed. The next step was to assemble the bioplastics and compare their strength, melting point, and antimicrobial properties.

After comparing the engineered bioplastics with oxo biodegradable plastic samples, the results showed that the engineered bioplastic was the most efficient. The bioplastic showed 89.94% biodegradation rate after 2 weeks. In terms of antimicrobial results, all the bioplastics showed significant effects against E. coli with garlic having the highest zones of inhibition of 25.28mm. The results showed it was more environmentally friendly and cost effective than the other plastics. The results also showed that this bioplastic can be a great alternative to petroleum-based plastics. I was able to utilize invasive algae which are detrimental to ecological balance and waste corncobs that have been a waste disposal problem by a lot of American farmers. Therefore, this project could be a solution for many environmental problems that our planet is facing.

RATIONALE

Current problems with plastics

Regular plastics are composed of major toxic pollutants. Negatively impact wildlife. Burning plastics contributes to global warming. Poses harm to the ocean and aquatic ecosystem.

Current solutions to plastic pollution:

PLA (Polylactic Acid)

PLA also known as "Corn Plastic" are lactic acid-based plastic.

Problem with PLA

For PLA to turn liquid and biodegrade, it requires 150-160^o C. (Barrett, 2020). PLA needs a special composting environment to biodegrade. These conditions can only be achieved in a large composting facility, which most individuals do not have access to. PLA also uses first generation food crops.

Oxo biodegradable plastic

Oxo biodegradables breaks down quicker than a regular plastic. However, it still requires the same fossil fuels during their manufacture and emit the same degree of **greenhouse gases** as conventional plastics. (Azios, 2007)

BACKGROUND RESEARCH

What are bioplastics?

According to Fazira (2014) and Marques (2017), bioplastics can be developed using natural sources such as starch and algae. Forbes.com supports bioplastics and believe that it will be the future of plastics. Furthermore, bioplastic industry is projected to have a 35% increase in the global market by 2030 and is worth about $324 Billion

Invasive algae

Caulerpa prolifera

Discovered in Newport Bay California last March, 2021. Can grow quickly and rapidly out-compete native species, including native eelgrass, and may be inedible to native marine herbivorous fish and invertebrates (Reports by California Department of Fish and Wild Life).

Undaria pinnatifida

Discovered in 2016, a highly invasive Asian algae that currently thriving in most central and southern California harbors including Ventura and Channel Islands Harbors (Reports by National Park Service). *Undaria pinnatifida* can be a good source of alginate which is used as emulsifier and thickener. (Lush.com)

WASTE CORN COBS

USA is the largest corn producer in the world (Prikhodko, 2018). Corn cobs are usually thrown away at big corn processing plants and are often considered a waste disposal problem (Sarian, 2016). On research conducted by Chatterjee et. al (2019), waste corn cobs were used to make bioplastics.

Possible benefit:

Will help American farmers in solving waste corn cobs problem.

Doesn’t use first generation crops.

STATEMENT OF THE PROBLEM

General Problem:

This research investigated the possibility of engineering antimicrobial bioplastics using invasive algae and waste corn cobs.

Specific Problems:

1. How can the engineered bioplastic be compared to other biodegradable plastics?
2. How effective are the engineered bioplastics in terms of their antimicrobial properties?

HYPOTHESIS

If starch from the waste corn cobs and alginate from invasive algae are combined with different plant extracts, then antimicrobial bioplastics can be engineered.

RESEARCH GOALS

This project has the following goals:

GOAL 1: Extract starch from waste corn cobs

GOAL 2: Create Sodium alginate from Undaria pinnatifida

GOAL 3: Extraction of *Caulerpa prolifera* and plants

GOAL 4: Assemble the antimicrobial bioplastics

METHODS

GOAL 1: Extract starch from waste corn cobs

Day 1-5: Sun drying and crushing of waste corn cobs

Day 6-7: Soak the dried corn cobs in water

Day 8: Blend the corn cobs using a blender and filter the blended solution using a cheesecloth

Day 9: Starch from the filtered solution was centrifuged to the bottom of the test tubes at 3000 rpm for 5 minutes

Day 10-16: Collect and place the starch in a digital incubator under 40^oC for 6 days to completely dehydrate

Day 17: Powder the dehydrated starch using a coffee grinder.

GOAL 2: Create Sodium alginate from Undaria pinnatifida

Preparation of Algae. Chop the dehydrated algae into pieces.

Alkalinization. Treat the chopped algae with NaOH solution containing 10% NaOH and boil it under 95^oC until it becomes jelly-like.

Separation. Initial separation/filtration of alkali alginate solution from its residue was done using cheesecloth. The filtered alginate undergone final separation using a centrifuge at 3000 rpm.

Alginic Acid Gel. Alginic acid gel was achieved by adding 5 mL of acetic acid to the filtered alginate**.**

Add Sodium carbonate. Add Na_2CO_3 to alginic acid gel and boil it to 90^oC to produce sodium alginate.

Dehydration of Sodium alginate. Dehydrate Sodium alginate in an incubator under 40^oC.

Powdered sodium alginate. Powder the sodium alginate using a coffee grinder and perform final dehydration using a digital incubator under 40^oC to remove excess water.

Goal 3: Extraction of *Caulerpa prolifera* and plants

Ethanol extraction and rotary evaporation. Plant samples including garlic, sage, and rosemary as well as Caulerpa prolifera were soaked in ethanol for a week. Rotary evaporation was performed to obtain pure extracts from the sample.

GOAL 4: Assemble the antimicrobial bioplastics

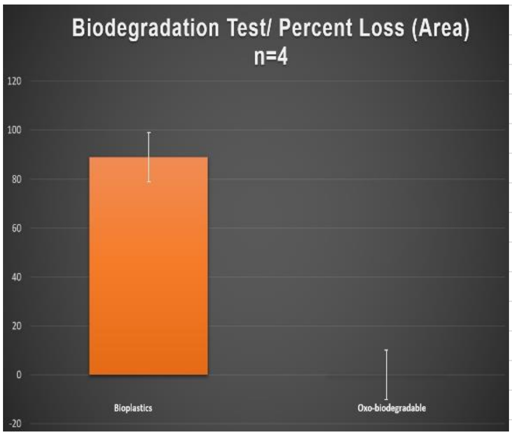
Preparation of Materials for Bioplastic Making. Prepare the Waste corn cob starch, sodium alginate from invasive algae, glycerin, distilled water and vinegar.

Heating. Heat the starch, sodium alginate, glycerin, distilled water, vinegar and on a hot plate. Wait for few minutes for the solution to cool down before adding the plant extract**.**

Shaping and Dehydrating. Place the heated mixture in a pan and place it inside a digital incubator under 60^{o} C to fully dehydrate.

RESULTS

BIODEGRADATION TEST: The engineered bioplastic and oxo-plastic was cut into pieces: 3X3 cm, 2x2 cm, 1x1 cm and 0.5x0.5 cm and buried in garden soil and placed outside for 2 weeks.



COST ANALYSIS				
	Unit Price	Price g/mL	Amount used	Price for amount used
Waste corncob	0	0	1.5 g	$0
Invasive Algae (If harvested in wild)	0	0	0.5 g	$0
Glycerin	3.88	0.022	0.5 mL	$0.0110
NaOH	39.4	0.00985	10 mL	$0.0099
NaCO3	4.12	0.0026	2 g	$0.0052
Vinegar	1.56	0.00082	5 mL	$0.0041
Distilled water	0.98	0.00026	10 mL	$0.0026
Total				$0.03275
Price per Square inch (Bioplastic)	111			0.00029505
Regular Petroleum Plastic (sq. in)	396			
TOTAL PRICE IF MADE WITH THE SIZE OF A REGULAR PLASTIC BAG				$0.1168

- Grocery stores normally charge $0.10 for a reusable/ compostable bag.
- Canvas bags cost about $1.25 -$2 a piece. (Nisperos N, 2016)
- My Engineered bioplastic would cost $0.12

If done in an industrial setting the price of the bioplastic will go down and could compete with the price of regular plastics.

Melting Point and Strength Test

Melting Point

SAMPLE	T1	T2	T3	T4	T5	AVERAGE
Bioplastic	85	87	92	88	83	87
Oxo-biodegradable	-	-	-	-	-	-

The results above present the temperatures where the plastics were turned into liquid. The bioplastic had an average melting point of 87^{o}C. On the other hand, the oxo-biodegradable did not melt, this is due to not sufficient heat.

Strength Test

Test Subjects	WEIGHTS (2 minutes)				
	5g	10g	20g	50g	100g
Bioplastic	✓	✓	✓	✓	x (1 min 12 sec)
Oxobiodegradable	✓	✓	✓	✓	x (1 minute 42 sec)
Cling Wrap	✓	✓	x (14 sec)	x	x

A 3x3 cm of Bioplastics, Oxo-biodegradable, and Cling wrap were prepared for strength test. Test subjects were hole punched and subjected with varying weights (5g, 10g, 20g, 50g and 100g)

Antimicrobial Test

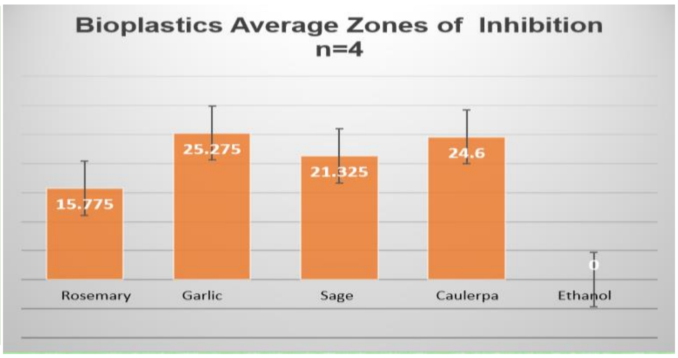
INTERPRETATION

(Johnson and Case, 1995)

Resistant- <10 mm

Intermediate- 11-15 mm

Susceptible- 16+mm



TEST 1: Modified Kirby-Bauer Sensitivity Assay

The antimicrobial bioplastics were tested on *E. coli*. Dilutions and inoculums were prepared following the 0.5 McFarland Standard. Bioplastics were placed under sterile paper discs. Mueller-Hinton plates were placed inside a digital incubator at 37°C for 8 hours.

ANOVA (Antimicrobial Effects of Bioplastics)						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1738.647	4	434.6618	150.2893	6.58E-12	3.055568
Within Groups	43.3825	15	2.892167			
Total	1782.03	19				

TEST 2: Inoculating lemons with fungi

Lemons were inoculated with fungi/mold and were observed for a week. All bioplastics were able to prevent the growth of the fungi. Control group showed fungal growth

DISCUSSION

Specific Problem 1: How can the engineered bioplastic be compared to other biodegradable plastics?

Based on the results of the experiments, the engineered bioplastic:

- had a better biodegradation rate (89.94%) as compared to oxo-biodegradable which did

  not biodegrade at all after 2 weeks.

- is cost effective ($0.11) and will compete successfully if done in an industrial facility.

- had a better melting point than the commercial plastic.

- almost as strong as the commercial plastic.

Specific Problem 2: How effective are the engineered bioplastics in terms of their antimicrobial properties?

Based on the results of the experiments, the engineered bioplastic:

- had a good antimicrobial effects against E. coli, with garlic having the highest average zones of

  inhibition of 25.28 mm followed by Caulerpa (24.6 mm), Sage (21.33mm), and Rosemary (15.78).

- Based on the results of the experiments, all bioplastics were able to prevent the growth of the fungi.

  Control group showed fungal growth

CONCLUSIONS

Based on the results of the experiments, my hypothesis is CORRECT! I was able to engineer a bioplastic that:

1. fully biodegradable and environmentally friendly (89.94% biodegradation rate in two

   weeks)

2. cost effective ($0.12)

3. had great antimicrobial properties.

Most importantly, I was able to engineer the first ever Antimicrobial Bioplastic from waste corncobs and invasive algae.

FUTURE PLANS

After doing this research, I have the following recommendations and plans:

1. Create different shapes of the engineered bioplastic (e.g. spoons, cups, and plates)
2. Improve the mechanical strength of the bioplastic by finding the best ratio between the materials used (Starch : Alginate)

POSSIBLE SOURCE OF ERRORS AND POINTS OF IMPROVEMENTS:

3. Perform melting point using a proper melting point equipment to get accurate results
4. Use silicone mold for better shaping of the plastic.

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TIP, Volume 17, Issue 1,Pages 92-96